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Is adding a new class of cones to the retina sufficient to cure color-blindness?

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New genetic methods have made it possible to substitute cone pigments in the retinas of adult nonhuman primates. Doing so influences the animals' visual abilities, demonstrating that the gene therapy was effective. However, we argue that no studies conducted so far have unambiguously demonstrated that the experimental animals have also acquired the ability to make new color distinctions. Simply put, it has been shown that animals that underwent the gene treatment can now—in addition to finding a red ball on a grayish background—find a green ball on a grayish background. However, it has not been shown that the animals can distinguish a red ball from a green one. For most people, that essential ability would be the primary reason for wanting to undergo a treatment for color-blindness in the first place, for instance, because their color-blindness currently prevents them from pursuing a career as a pilot or firefighter. It is important to point out such possible limitations of gene therapy for color-blindness to avoid unwarranted expectations in both clinicians and patients. To explain the origin of our concerns, we simulate how replacing the pigment of some cones is expected to influence the outcomes on the behavioral test used so far. The simulations show that this test does not provide conclusive evidence that the animals acquired the ability to make new chromatic distinctions. In our view, it is therefore premature to claim that human color-blindness can be cured through gene therapy. We propose a test that would provide more conclusive evidence of fundamentally altered color vision after gene therapy.

Introduction

Introducing a functioning third pigment into the cones of a dichromatic retina must influence the organism's vision because cones with a different functional pigment will respond differently to the light falling on them. The critical question is whether the altered spectral sensitivity will enable the organism to make distinctions based on color (i.e., chromatic distinctions) that it previously could not.

A study by Mancuso et al. (2009) with squirrel monkeys (*Saimiri sciureus*) has led to far-reaching claims to success in restoring normal color vision through gene therapy (Bennett, 2009; Conway et al., 2010; Liu, Tuo, & Chan, 2011; Mancuso, Mauck, Kuchenbecker, Neitz, & Neitz, 2010; Shapley, 2009).[1] The critical issue is the suggestion that the monkeys could make new higher-dimensional (i.e., trichromatic rather than dichromatic) color distinctions when provided with a third kind of cone sensitivity. To evaluate whether this can really be concluded from the existing evidence, we examine whether the behavioral test that was used required such a higher-dimensional color percept (Jameson et al., 2001; Jordan, Deeb, Bosten, & Mollon, 2010; Zaidi, Marshall, Thoen, & Conway, 2014). It is evident that the monkeys that received a new cone pigment could subsequently make certain distinctions that they could not make before the treatment. However, is this just because they see the world differently with the modified cones—just as we see the world differently if we place a colorful filter in front of our eyes—or could the monkeys really distinguish between more colors (Makous, 2007)?

Before treatment, the dichromatic monkeys were able to distinguish between colors by comparing the stimulation of middle (M) and short (S) wavelength sensitive cones. Consequently, they could detect targets on a gray background by their color if the ratio of M and S cone stimulation was different for the target than for the background (resulting in the detection thresholds shown by the green curve in Figure 1; see also

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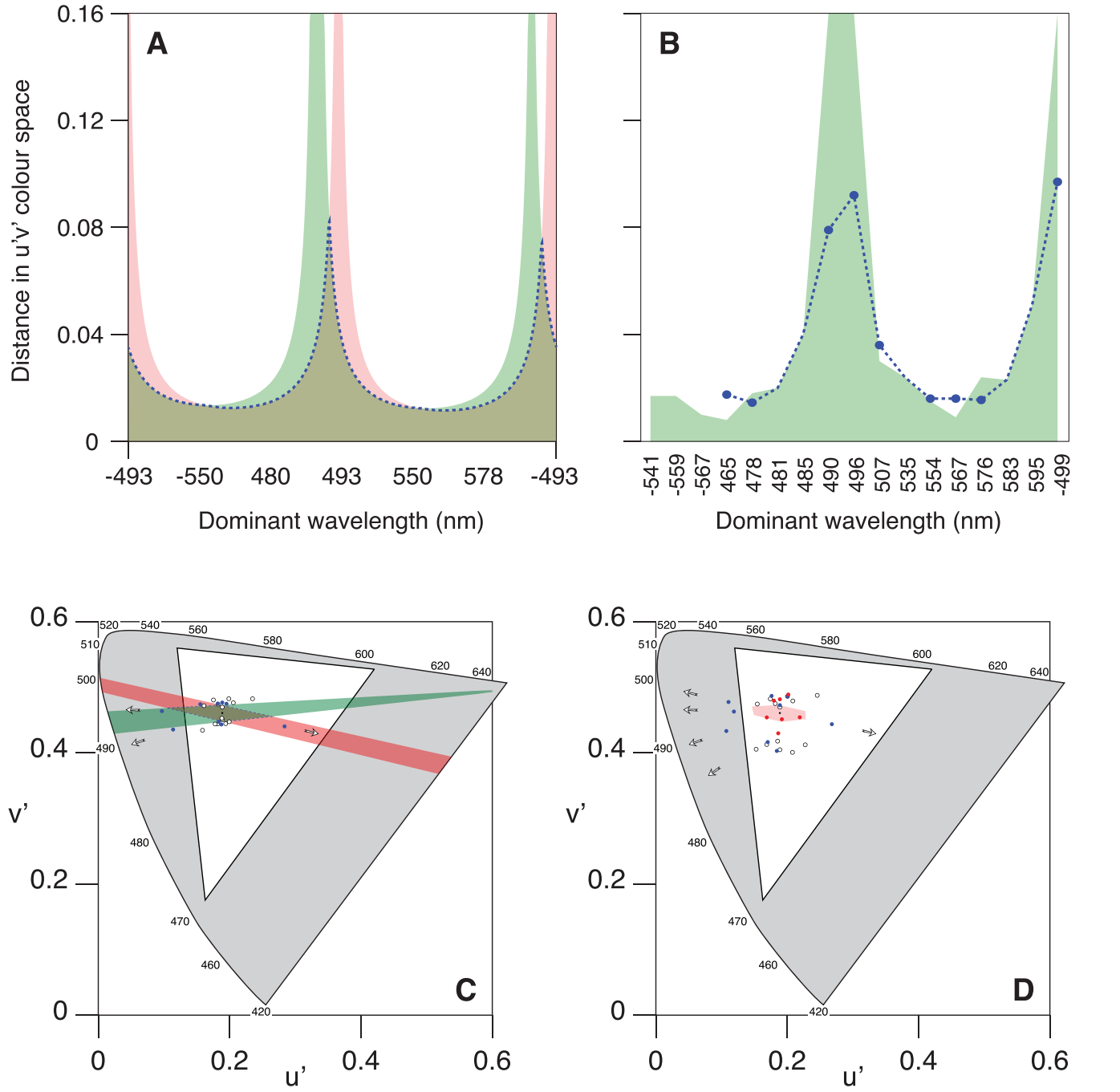

Figure 1. Simulation that explains why the fact that some targets could only be detected after gene therapy does not prove that there was a change in how cone outputs were interpreted. (A) We assume that the target cannot be detected when the ratio between either L and S cones (red; deuteranopes) or M and S cones (green; protanopes) differs by less than 15% between the target and the "gray" background. The blue curve corresponds with the expected saturation threshold if the target can be detected when either of the ratios exceeds 15%. Note that there is still a peak in the threshold but that near 490 nm the target is no longer undetectable. Thresholds are expressed as vector lengths from the "gray" origin. (B) The green region shows the saturation values at which a squirrel monkey could not detect the target prior to the therapy (data from figure 3c of Mancuso et al., 2009). The blue dots and curve show the same monkey's saturation threshold after the gene therapy. After the therapy, the monkey can detect colors at 490, 496, and −499 nm whereas prior to this it could not (as predicted by the blue curve in A). (C) The same simulations and results presented in u'v' color space. The white triangle indicates the region that can be rendered on one of our CRT screens, indicating the approximate distances that were available for the color tests (a similar range is shown in Mancuso et al., 2006). The small black dot is the gray origin. The red and green regions and the blue dashed lines reproduce the values from (A). The symbols reproduce the values

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←

from (B). Open symbols indicate thresholds before treatment with arrows indicating that no threshold was found in a given direction. Blue symbols indicate thresholds after treatment. (D) Thresholds for the other treated monkey (data from figure 3b of Mancuso et al., 2009) and for a trichromatic monkey (data from figure 2e of Mancuso et al., 2009) in the same format. The pink region indicates the area in which none of the cone contrasts (including that between L and M cones) is larger than 15%.

figures 2b and c in Mancuso et al., 2009). Mancuso et al. (2009) introduced a long (L) wavelength sensitive pigment into some of the monkeys' M cones. If the pigment had been modified in all M cones without any change in the way in which the signals from those cones are interpreted, the treatment would have simply shifted the colors that cannot be distinguished from gray from being ones that maintain the M-to-S cone ratio to being ones that maintain the L-to-S cone ratio (red curve in Figure 1; this is also explained in Mancuso et al., 2009, and in Shapley, 2009).

Because the pigment was only changed in a fraction of the cones, the monkey might be able to detect targets that are invisible to the unchanged cones with the modified cones and vice versa. The monkeys might therefore be able to detect a colored target when *either* the ratio of L and S cone stimulation *or* the ratio of M and S cone stimulation is different from that of the gray background (simulated thresholds indicated by the dashed blue curve in Figure 1A). To illustrate that this alone could account for how performance changed in the task that was used to evaluate the gene therapy's influence on the monkeys' color vision, in the absence of any further changes in neuronal connectivity, we simulated the possible appearance of several targets to eyes with various combinations of cones. Simulation details are provided in the Methods section at the end of this paper.

Results and discussion

The simulated threshold for a monkey that is able to detect a colored target when *either* the ratio of L and S cone stimulation *or* the ratio of M and S cone stimulation is different from that of the gray background is strikingly similar to one of the monkey's performance (compare the dashed blue curve in Figure 1A to the dashed blue curve in figure 3c of Mancuso et al., 2009, reproduced in our Figure 1B). Note that the simulated performance is a direct consequence of replacing the pigment in some cones. It does not require any changes to postreceptoral processing. The only requirements are that the new pigment is functional and that the monkey detects the target if *either* the original comparison between M and S cones *or* the new comparison between L and S cones is different. The comparison between stimulation of M or L cones and stimulation of S cones must be made locally, but some averaging of signals from L and M cones is likely to occur before the combined signal is compared with signals from S cones. This may explain why the improvement in the threshold after treatment is slightly smaller than our simulations predict.

Figure 1C shows the same data as Figure 1B plotted in u'v' color space. We see some discrepancies between the directions in which the monkey cannot detect targets before treatment (arrows, especially the one pointing to the lower right) and the prediction based on human cones (green area), but overall, the pattern is described quite well by a threshold of 15% difference in cone stimulation. After treatment, the monkey performed slightly better in some directions (compare blue symbols to open symbols) but no better than predicted from a combined ability to detect targets that are visible to either a comparison of L and S cones or M and S cones (dashed blue line, overlap between red and green areas).

The similarity between our simulations and the other monkey's performance is less striking, but the overall pattern is comparable (dashed blue curve in figure 3b of Mancuso et al., 2009; open and blue symbols in Figure 1D). That monkey generally performed less well for bluish colors (lower values of v') both before and after treatment. A trichromatic control monkey had a consistently low threshold without the characteristic peak near 490 nm (dashed blue curve in figure 2e of Mancuso et al., 2009; red symbols in Figure 1D). This is because trichromatic monkeys directly compare the signals from their L and M cones.

Hence, there is no doubt that the genetically treated monkeys have become *three-photopigment individuals* (in analogy to the definition of Jameson et al., 2001, for tetrachromacy). They also presumably have ganglion cells that compare L and S cone stimulation as well as ganglion cells that compare M and S cone stimulation and probably also ganglion cells that are excited and inhibited differentially by L and M cones (Shapley, 2009). The question is whether the information that these ganglion cells provide results in the ability to make new—higher-dimensional—color discriminations. This is both an intriguing scientific question and a clinically relevant one because if nothing has changed in the postreceptoral connectivity, the monkeys will probably just judge some surfaces' colors slightly

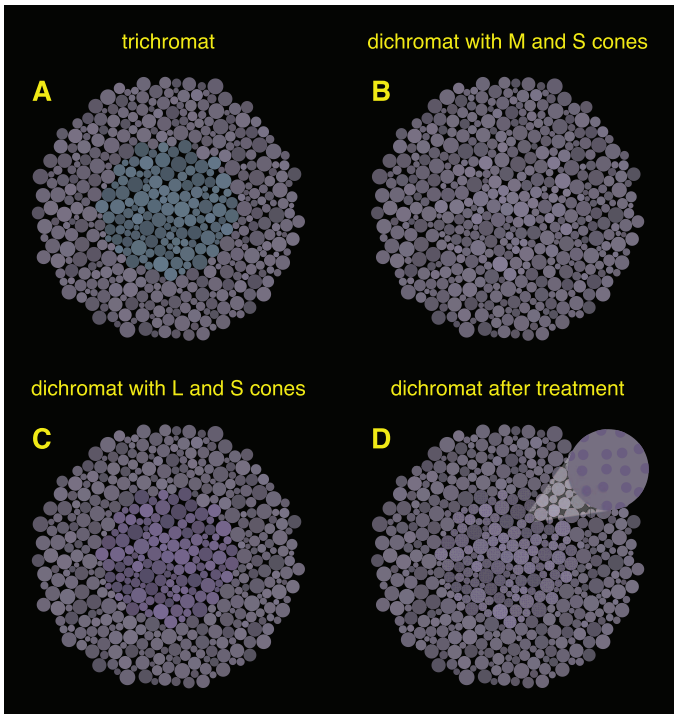

Figure 2. Simulation of the possible appearance of a "bluish" target on the gray background. (A) Stimulus at the trichromatic detection threshold. (B) How the stimulus at the detection threshold after the gene therapy might look to a monkey with only M and S cones. (C) How it might look to a monkey with only L and S cones. (D) How it might look to a monkey in which the M cone photopigment has been replaced by the L cone photopigment in 25% of the cones (represented by the tiny circular regions inside the disks that make up the target) with no further changes in the way in which signals are interpreted.

differently but within their original—dichromatic—range of distinctions. However, they now have differences in color-opponent signals at different retinal locations. If they were to also change postreceptoral connectivity, the monkeys could—theoretically—gain the ability to make new chromatic distinctions beyond the dichromatic range.

To help explain the distinction between being able to detect targets that they previously could not and being able to discriminate between additional colors, we simulated what a colored target disc (490 nm) on a gray background might have looked like for the treated monkeys (Figure 2). Note that the purpose of our simulations is to demonstrate to what extent the target differs from the background. We do not wish to make any claims as to whether this is really what it would look like to the monkeys (either treated or not).

Mancuso, Neitz, and Neitz (2006) used a behavioral detection task to demonstrate that the monkeys were using their modified cones. In this task, monkeys had to indicate the location of a target that differed in chromaticity from the background. Figure 2A shows about what this target looked like at the trichromatic control monkey's detection threshold. Figure 2B shows the possible appearance of a more saturated target of the same color to a dichromatic monkey lacking L cones. Figure 2C shows the possible appearance of the same target to a dichromatic monkey lacking M cones. Although the target is less clear in Figure 2C than in Figure 2A, it is definitely visible unlike the target in Figure 2B (note that the critical issue is that the target differs in color from the background irrespective of whether this is really what it looks like to a dichromat).

Figure 2D simulates the target for a monkey in whose retinae some of the M cones were replaced by L cones without any further changes in neural circuitry. The main thing to note is that—although the target cannot be detected by comparing M and S cone signals—parts of it are visible because part of the retina is now comparing L and S cone signals. As a result, the target can be detected despite the range of considered colors not having changed. The detection is based on the tiny circular regions inside the disks that form the target, which represent the regions in which L and S rather than M and S cone stimulation is compared.

It is unlikely that the target appears "textured" to the monkey as it does in Figure 2D because the texture in the figure is caused by the differences between the cones, so it moves with the eyes rather than sticking to the surface. However, the "nonuniformity" itself could be detected (Makous, 2007). The nonuniformity is unlikely to be experienced as such, much as the spatial distribution of the different kinds of cones in the retinal matrix is not perceived (Jacobs & Nathans, 2007). Nevertheless, the nonuniformity may result in a signal that depends on a surface's color and on gaze in the same way as do regular color signals. In what way would using such a nonuniformity cue differ from true (although not necessarily conventional[2]) color vision? The minimal requirement for such a nonuniformity cue to allow the monkey to make additional chromatic distinctions is that the monkey must be able to relate specific nonuniformities in the signal to the underlying stimulated cone types. Just knowing that L and M cones are not stimulated equivalently is not enough.

Figure 3 shows one way in which one could proceed to demonstrate that genetic treatment allows monkeys to make new chromatic distinctions. The targets in Figure 3A and B do not differ in S cone stimulation, but they do differ in L and M cone stimulation. Both targets will be visible to a genetically treated monkey. They differ in whether the treated or the untreated cones respond more strongly (the positions of signals from the treated cones are indicated by tiny dots). Simulations of how this might look through local comparisons of either only L and S cones or only M and S cones (as in Figure 2D) are shown in Figure 3C

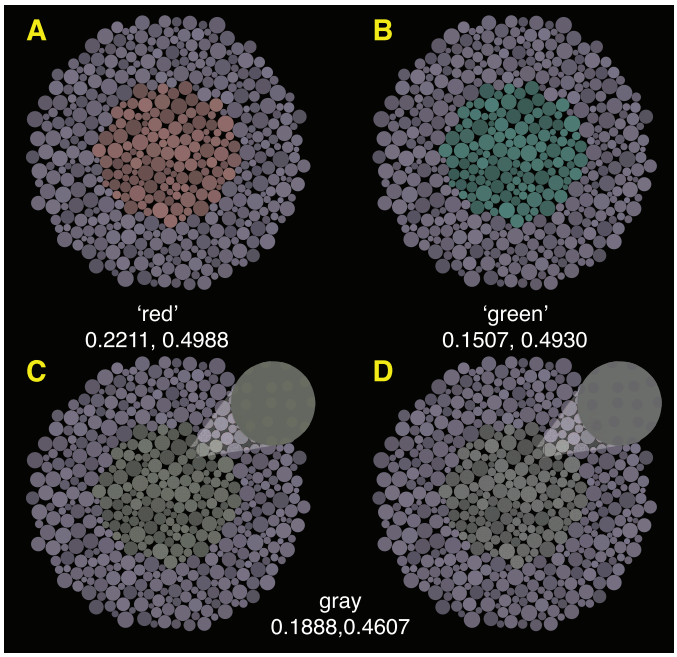

Figure 3. A very basic test of whether the gene therapy–treated monkeys have developed the ability to make new chromatic distinctions is to determine whether they can distinguish between additional stimulation of modified and of unmodified cones. A possible way to do so would be to try to train them to distinguish between "red" (A) and "green" (B) targets that would appear very similar to a monkey that combines two different kinds of dichromacy as illustrated in Figure 2D rather than directly comparing L and M cones. Without considering which cones were modified, the "red" target (C) would be indistinguishable from the "green" one (D), especially if the luminance and saturation of the targets were varied randomly across trials. By considering which cones were modified, the colors could readily be distinguished on the basis of the polarity of the difference between modified and unmodified cones (as shown by the relative colors of the tiny dots; see enlargements in C and D).

and D. The two "patterns" that arise from comparing S cones with both L and M cones look very similar, but note that they are actually complementary in terms of the positions of each of the colors. If the monkeys can (learn to) distinguish between these two patterns, they must have access to information about the identities of the two signals—rather than only being able to detect the nonuniformity per se—and would have acquired the ability to make a new chromatic distinction. One would probably want to vary the saturations of the colors across trials to prevent the monkeys from responding based on subtle differences in contrast.

Note that we are searching for evidence that the monkeys can distinguish between the targets on the basis of what we normally refer to as their color. We are not trying to predict the sensation that would come with this for the monkeys. If one would want to try to conclude something about the monkeys' percepts rather than only about their ability to make new chromatic distinctions, a possible way to proceed could be to examine how conspicuous they find differences in color in comparison to differences in orientation or size in a visual search task (see Brenner, Cornelissen, & Nuboer, 1990).

Our claim is that the current evidence does not demonstrate that the monkeys have learned to make new, higher-dimensional chromatic distinctions. This does not diminish the fact that the newly developed genetic technique (Jacobs, Williams, Cahill, & Nathans, 2007; Mancuso et al., 2009) provides exciting new ways to study whether and, if so, how new and perhaps even unconventional neural circuits for color vision are established. It is still not clear why many aspects of vision only develop with enough exposure during a critical period early in life (Blakemore, 1976; Blakemore & Cooper, 1970; Cynader & Chernenko, 1976; Rauschecker & Singer, 1981; Wiesel & Hubel, 1963) whereas color vision does not appear to require such exposure (Brenner et al., 1990; Brenner, Schelvis, & Nuboer, 1985; Di, Neitz, & Jacobs, 1987). Comparing the color vision and neuronal activity of dichromatic and trichromatic monkeys and of monkeys that were born dichromatic but were made trichromatic later in life could provide insight into this issue. However, at present, it is premature to conclude that new *higher-dimensional color* skills arise automatically when one introduces a "missing" pigment in the primate retina. It is therefore also premature to claim that human color-blindness can be cured through gene therapy.

Methods

Our modeling is based on human color vision. We used standard procedures to convert colorimetric data to human L, M, and S cone values (based on the Vos-Walraven human cone spectral sensitivity functions; for details of the transformations see appendix A of Granzier, Brenner, & Smeets, 2009). With these values, we could determine by how much colors had to differ for the selected cone ratios to differ by a certain amount (the selected threshold). We chose a cone ratio threshold of 15% to distinguish between targets that could and could not be detected because this nicely matched the performance of the second monkey in Mancuso et al. (2009) before treatment. Results are presented in terms of saturation (distance from the chromaticity of the grey background at [u' = 0.1888, v' = 0.4607]), as in the original article, as well as as positions in u'v' color space.

To illustrate our arguments, we also simulated the possible appearance of several targets to eyes with various

combinations of cones (Figures 2 and 3). To simulate the appearance to a dichromatic eye without L cones, we took the values that we determined for how each disk in the image would normally stimulate L, M, and S cones and discarded the values of the L cones. In order to render an impression of what the target might look like in the absence of L cones, the color of the surface must be set to give a value of L cone stimulation that does not add any diversity in color to the scene. One way to achieve this is to set the value of L cone stimulation to a constant proportion of the stimulation of the M cone. The proportion that we chose was the same proportion as the proportion of L relative to M cone stimulation by the gray background (for a similar approach, see Viénot, Brettel, Ott, Ben M'Barek, & Mollon, 1995).

An equivalent procedure was used to render an impression of what the target might look like in the absence of M cones. Note that the purpose of these simulations is to demonstrate to what extent the target differs from the background. We do not wish to make any claims as to whether this is really what it looks like to the corresponding dichromats. For an eye in which some of the L cones were replaced by M cones, we assume that the color is determined locally on the basis of the cone comparisons that were used to make chromatic distinctions before the cone replacements, without considering whether these local comparisons now involve L and S cones or M and S cones.

To illustrate the limitation of the original behavioral test, we simulated the possible appearance of a "bluish" target on a gray background near the monkey's detection thresholds. We chose this target color (equivalent dominant wavelength of 490 nm) because it lies near the protan confusion line described in Mancuso et al. (2006). Variations in the luminances and sizes of the small disks that make up the target are approximately as used in the experiments of Mancuso et al. (2009). To render the appearance for a trichromatic monkey (Figure 2A), we simulated the stimulus at the trichromatic detection threshold (saturation of 0.02, estimated from figure 2e of Mancuso et al., 2009: [u' = 0.1689, v' = 0.4591]). To simulate the possible appearance of the stimulus for dichromatic monkeys missing the L cone photopigment (Figure 2B) or M cone photopigment (Figure 2C), we simulated what it might look like at the detection threshold after treatment (saturation of 0.085, estimated from figure 3b of Mancuso et al., 2009: [u' = 0.1041, v' = 0.4537]). To simulate how it might look to a treated monkey, we assumed that the M cone photopigment has been replaced by the L cone photopigment in 25% of the cones (represented by the tiny circular regions inside the disks that make up the target in Figure 2D). This simulation shows that the target may be *detected* due to the local comparisons with L cones without the monkey being able to make any new chromatic distinctions.

To illustrate an alternative test that would require an ability to make new chromatic distinctions, we also simulated the possible appearance of "red" and "green" targets (Figure 3). If the task is to discriminate between red and green targets and luminance and saturation are varied so that they cannot be used reliably, the very least that the monkey would have to have access to in order to make the distinction is which local comparisons involve L and S cones and which involve M and S cones. Of course, if L and M cones are compared directly, the monkey will be able to make more chromatic distinctions. Conversely, if local comparisons are made without considering whether or not individual cones have been modified, red and green targets will look very similar (Figure 3C and D).

Colors were rendered on a calibrated monitor, but the reproduction in the figures is not calibrated, so the figures only show an approximation of the contrasts. Using standard human cone data to approximate the treated monkeys' sensitivities, together with the loss of color calibration when publishing the figures, means that the colors in the figures do not precisely match what the monkey might have seen. However, we believe that providing such approximate simulations helps illustrate the limitations of the original behavioral test used to demonstrate trichromacy (as described in Mancuso et al., 2006).

Keywords: color vision, gene therapy, color-blindness, development, simulations

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Footnotes

[1] See also: http://www.neitzvision.com/content/genetherapy.html and http://investors.avalanchebiotech.com/phoenix.zhtml?c=253634&p=irol-newsArticle&ID=2028354

[2] For example, monkeys reared under continuously changing monochromatic illumination appear to develop a functional yet unconventional kind of color vision (Brenner & Cornelissen, 2005; Sugita, 2004).

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